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Effects of two feed additives on performance, energy digestibility, and body composition of first-cycle laying hens fed two concentrations of dietary energy

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DEDICATION

In loving memory of Janie Molenbrock and Joseph Hacker.

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NOMENCLATURE

HE	High Energy
LE	Low Energy
ME	Metabolizable Energy
SSF	Solid-State Fermentation
MRF	Mannose Rich Fraction
MOS	Mannan Oligosaccharide
AMEn	Nitrogen Corrected Apparent Metabolizable Energy
AFP	Abdominal Fat Pad
DXA	Dual-Energy X-ray Absorptiometry
EU	Experimental Unit
HDEP	Hen-Day Egg Production
FI	Feed Intake
E:F	Egg to Feed Ratio

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ABSTRACT

Three experiments were conducted to determine the effects of feed additives on the performance, energy digestibility, and body composition of first-cycle laying hens fed two concentrations of dietary energy. The first and second experiments were 8 wk in duration. The first experiment contained two dietary energy levels (2,750 and 2,850 kcal/kg) with or without the addition of a mannose rich fraction (**MRF**) from a specific strain of yeast. As expected, no differences in performance parameters including egg production, egg weight, and egg mass were detected, although increased dietary energy, but not MRF resulted in increased total and percentage of hen fat mass as determined by DXA analysis. A similar response to dietary energy was observed for abdominal fat pad weight, but significance was not achieved (p = 0.12). Mannose rich fraction treatment increased nitrogen corrected apparent metabolizable energy (**AME_n**) but the increased dietary energy did not appear to be stored as increased fat within the hen. The second experiment contained the same two dietary energy levels (2,750 and 2,850 kcal/kg) with or without the addition of an *Aspergillus niger* derived product created from solid-state fermentation (SSF) containing various enzyme activities. There was a significant SSF by dietary energy interaction (p = 0.02) on fat mass of hens in which reduced dietary energy without SSF resulted in reduced fat mass, but reduced dietary energy with SSF resulted in increased fat mass. Both dietary energy and SSF resulted in a significant ($p < 0.01$) increase in AME_n. Again, no differences in performance parameters including egg production, egg weight, and egg mass were detected in experiment 2. In these two 8 wk experiments, laying hen performance parameters were unaffected, despite, significant differences in body composition and dietary AME_n suggesting that short term performance is a poor indicator of energy and feed additive status The third experiment was 16 wk and hens were fed two

concentrations of dietary energy (2,850 and 2,950 kcal/kg) with three SSF inclusions (0%, 0.2%, and 0.4%). A significant effect of SSF on HDEP ($p \leq 0.01$) was seen in which the 200 SSF and 400 SSF fed birds resulted in a 1% decrease in egg production compared to the control hens. The solid-state fermentation product had a significant ($p = 0.02$) effect on egg mass in which birds fed the 200 SSF and 400 SSF diets produced 1 g less egg mass than the control hens (due to lower egg production and not egg weight). The solid-state fermentation product also had a significant ($p = 0.02$) effect on feed efficiency in which laying hens fed the 200 SSF diet consumed 9 g/kg more feed to produce an egg than the control hens. There were significant ($p \leq 0.01$) effects of dietary energy on 16 wk fat mass and AFP in which the high energy (HE) fed birds contained more fat than the low energy (LE) fed birds. There was a significant ($p \leq 0.01$) interaction of energy and SSF in which the 0.2% SSF treatment resulted in the highest AME_n in the high energy diet, but the 0.4% SSF treatment resulted in the highest AME_n in the lower energy diet. During the 16 wk experiment SSF treatment improved AME_n, but negatively impacted egg production, and did not influence body composition.

CHAPTER 1

GENERAL INTRODUCTION

Feed costs account for approximately 70% of total poultry production costs (Barletta, 2010). Although poultry are efficient converters of feed to body weight gain or egg production, they cannot digest 15-25% of the feedstuffs they consume because of anti-nutritional factors that may hinder digestion and/or remain indigestible to the endogenous enzymes present within the bird (Paloheimo et al., 2010). Anti-nutritional factors present in corn and soybean meal can interfere with the bird's feed utilization and may affect health and production. The anti-nutrients in corn include nonstarch polysaccharides, phytic acid, enzyme inhibitors, and resistant starches. Soybean meal contains protease inhibitors, nonstarch polysaccharides, lectins, phytic acid, saponins, phytoestrogens, anti-vitamins, and allergens (Cowieson, 2005; Francis et al., 2001). Therefore, it is important to understand the effects feed additives have on energy metabolism of poultry and how that energy is being utilized for maintenance, production, or storage.

One of the limitations of currently published research protocols using a laying hen model to explore reductions of small amounts of dietary energy is the bird's ability to adjust feed intake or even metabolism to account for those differences in dietary energy without altering short-term egg production. Laying hens utilize dietary energy in three ways: maintenance, production, and storage. The main result of energy consumption is typically split between productive and maintenance requirements, but storage, a third option, is often overlooked in laying hen energy evaluation. Dual-energy x-ray absorptiometry has the capability to quantify differences in energy storage in laying hens over an experimental period.

Therefore, the two-step hypothesis for this research was:

1) Differences in body composition, abdominal fat pad, and nitrogen corrected apparent metabolizable energy would be detected when laying hens were fed two concentrations of dietary energy and/or the addition of feed additive, mannose rich fractions or solid-state fermentation product, over an 8 wk feeding period.
2) Differences in body composition, abdominal fat pad, and nitrogen corrected apparent metabolizable energy would be detected when laying hens were fed two concentrations of dietary energy and/or the addition of a solid-state fermentation product over a 16 wk feeding period.

The first objective of our research was to evaluate the addition of a feed additive, mannose rich fractions or a solid-state fermentation product, on body composition, abdominal fat pad, and nitrogen corrected apparent metabolizable energy of laying hens fed diets differing in energy concentration over a short-term 8 wk experiment. The second objective of this research was to evaluate two inclusion levels of a solid-state fermentation product, Allzyme SSF, on body composition, abdominal fat pad, and nitrogen corrected apparent metabolizable energy of laying hens fed diets differing in energy concentration over a 16 wk experiment.

CHAPTER 2

REVIEW OF THE LITERATURE

Introduction

Feed costs account for approximately 70% of total poultry production costs (Barletta, 2010). In the United States, the two major poultry feed ingredients are corn and soybean meal. In 2012, the American Soybean Association estimated the use of soybean meal in poultry production to be 49% of the total used in livestock production. Unfortunately, anti-nutritional factors present in corn and soybean meal can interfere with the bird's feed utilization and may affect health and production. Soybean meal has a number of anti-nutrients present including protease inhibitors, nonstarch polysaccharides, lectins, phytic acid, and saponins (Francis et al., 2001). The anti-nutrients in corn include nonstarch polysaccharides, phytic acid, enzyme inhibitors, and resistant starches (Cowieson, 2005). New technology such as the addition of feed additives can reverse the inefficiencies caused by these anti-nutrients. The objective of this literature review is to build the necessary foundation for enzyme and mannan oligosaccharide products and understand how they have influenced short-term experiments.

Energy

Grain prices today are being driven by the increasing demand for energy as consumption of corn, soybean meal, wheat, and barley have increased around the world (Barletta, 2010). Therefore, understanding energy digestion, metabolism, and ultimately utilization patterns of the laying hen has become important in minimizing feed costs while still maintaining and improving

feed efficiency and energy utilization (Pardue, 2010). Laying hens metabolize energy in three ways: maintenance, production, and storage (Sakomura, 2004).

Sakomura (2004) defines maintenance as the amount of energy required to balance catabolism and anabolism while having energy retention around zero. The National Research Council defines it as the body's metabolizable energy needs in order to maintain the body's normal functions and moderate activity (NRC, 1994). The requirements for energy maintenance have been calculated by utilizing calorimetric measurements, feeding trials, and finally by using a regression equation of energetic balance components. Factors that can affect an animal's energy metabolism include body weight, age, body composition, size of organs, and production or growing stage (Blaxter, 1989).

Another priority in energy partitioning in poultry is production, whether it is production of eggs in laying hens or meat in broilers and turkeys. Partial efficiencies for either laying hens or broiler breeder hens are hard to determine so instead some researchers prefer to put efficiency for growth and egg production together. However, Sakomura (2004) calculated partial efficiencies for energy utilization in laying hens for growth and egg production were 65% and 62% respectively. Determining the same thing for broiler breeder hens they found a slight variation in which energy utilization for growth after lay was 47% and egg production was 64%. These partial efficiency values are important because it is difficult to quantify the energy required for growth and egg production separately in laying hens.

Laying hens store energy only after their maintenance and production requirements are met. In poultry maintenance requirements are met first, followed by lean protein accretion, and lastly fat accretion (Kielanowski, 1965). If maintenance requirements are not satisfied the bird

must catabolize stored nutrients to replace those in the diet (Sakomura, 2004). The body composition of a bird changes with age, body weight, genetics, and diet (Kielanowski, 1965).

A laying hen experiment conducted by Valkonen et al. (2008) examined the effects of dietary energy content on egg production and egg quality of two various caging systems (8-hen furnished cages vs. 3-hen conventional cages). Two dietary energy concentrations (low: 2,342 to 2,414 kcal/kg and high: 2,581 to 2,629 kcal/kg) were fed during three feeding phases of 20, 16, and 16 weeks respectively. This experiment utilized 1,088 Lohmann Selected Leghorn and hens were allowed *ad libitum* access to feed. Hens that were fed the low energy diet consumed 8-9 g/hen/d more ($p < 0.01$) than hens fed the high energy diet. Hens consuming the low energy were also found to produce approximately 2% fewer eggs per day ($p < 0.05$) than birds on the high energy diet (Valkonen et al., 2008). Bohnsack et al. (2002) conducted a 12-week experiment using 560 Hy-Line W36 laying hens fed seven various levels of ME obtained by utilizing corn oil (2,783, 2,891, 2,996, and 3,089 kcal/kg) or poultry fat (2,881, 2,975, and 3,059 kcal/kg) at 0, 2, 4, and 6% inclusion levels and concluded that feed intake did not increase when dietary ME was reduced from 2,996 to 2,783 kcal/kg. D'Alfonso et. al (1996) demonstrated that various concentrations of dietary ME (2,580, 2,814, and 3,009 kcal/kg) in De-Kalb XL laying hen diets over a 7 wk experiment saw no significant differences in egg production, egg mass, and body weight. Feed intake linearly decreased with increasing dietary energy levels indicating that hens consume feed until there energetic demands are met (D'Alfonso et. al, 1996).

Jalal et al. (2006) conducted a 15 wk experiment utilizing Hy-Line W36 laying hens looking at three dietary energy levels (2,800, 2,850, and 2,900 kcal/kg) and their effects on performance and AME_n. Dietary energy level did not significantly affect feed intake, HDEP, egg mass, and egg weight. They found that hens fed the 2,900 kcal/kg diet had significantly greater

AME_n as compared to the 2,850 and 2,800 kcal/kg diets with differences of 107 and 118 kcal/kg, respectively. This was expected since diets were formulated to have different ME levels. Harms et al. (2000) conducted an experiment using 120 of each four commercial laying hen strains (Hy-Line Brown, Hy-Line W98, Hy-Line W36, and DeKalb White) to quantify response to changes in dietary energy concentration. Hens were fed from 36 to 44 weeks of age and were fed one of three dietary energy levels which included low (2,519 kcal/kg), control (2,798 kcal/kg), and high (3,078 kcal/kg). Results indicated that laying hens fed the low energy diet consumed 8.5% more feed than hens fed the control diet. On the other hand, hens offered the high energy diet consumed 1.5% less feed than hens fed the control diet. Together these results show the four specific strains of laying hens are more sensitive to lowering the energy in the diet than increasing it. The hens are more sensitive to changes in dietary energy at lower dietary energy concentrations. Both the Hy-Line Brown and Hy-Line W98 were found to be more sensitive to changes in dietary energy than Hy-Line W36 and DeKalb White. This is most likely due to the Hy-Line Brown and Hy-Line W98 being larger birds than either the Hy-Line 36 and DeKalb White therefore their requirement for maintenance energy is higher. Differences in dietary energy levels did not affect egg production. However, egg weight significantly increased by 2% with the high energy diet (Harms et al., 2000).

An 8 wk experiment was performed in which Hy-Line W36 hens approximately 80 wks of age were fed two various concentrations of energy, 2,519 and 2,798 kcal/kg respectively, equating to a 10% increase in energy. Results indicated that laying hens feed intake and egg production remained unaltered (Harms and Russell, 2004). Egg production was not affected by differences in dietary energy over short-term experiments.

Egg production in laying hens only accounts for part of the energetic balance. Therefore performance responses alone are not sufficient to understand energy utilization. Since egg

production along with other performance parameters seemed insensitive to short-term experiments, body composition became an area of interest to detect and quantify differences in dietary energy. Murugesan and Persia (2013) conducted a 12 wk experiment to evaluate energy utilization in 60 Hy-Line W36 laying hens fed two ME concentrations (2,880 and 2,790 kcal/kg) on two different feeding programs (restriction fed and *ad libitum*). There was a significant ($p \leq 0.01$) effect on feeding regimen in which feed intake in the *ad libitum* group consumed 10 g/hen/d more than the restricted group. Hen-day egg production in the *ad libitum* fed group was 3.5% higher than the restricted group ($p \leq 0.01$). Effects were also seen in egg mass, body weight, and abdominal fat pad weight in which the *ad libitum* group again was higher than the control by 3.5 g/hen/d for egg mass, 80 g heavier in body weight, and 23.8 g/hen in abdominal fat pad weights. The energy intake differences between the two diets did not hinder production or maintenance but did reduce the energy stored in the fat pad of the hens on the lower ME diet. These results indicate that over a short period of time in Hy-Line W36 laying hens energy is first partitioned to production and maintenance then if excess is left it goes to storage within the fat pad (Murugesan and Persia, 2013).

In summary, over short-term experiments differences in dietary energy had very little if any influence on egg parameters such as egg mass, egg weight, and HDEP. Feed intake was impacted by the amount of energy in the diet in which birds adjusted feed intake by energy concentration in the diet. However, none of these experiments looked at body composition of laying hens with the exception of Murugesan and Persia (2013) who looked at abdominal fat pad. Future experiments especially looking at the various effects of different energy levels should investigate how dietary energy influences body composition in laying hens.

Enzymes

Endogenous digestive enzymes act on complex nutrients such as proteins, starches, and fats in order to break them into smaller compounds. Although poultry are efficient converters of feed to gain, they cannot digest 15-25% of the feedstuffs they consume because of anti-nutritional factors that may hinder digestion and/or remain indigestible to the endogenous enzymes present within the bird (Paloheimo et al., 2010).

Hemicellulose is the second most common structural polysaccharide found in plants with the four types being arabinans, galactans, glucomannans, and xylans. The two main types include xylans and glucomannans. Xylanases or endoxylanases, are responsible for cleaving the β-1,4 linkages of xylan polymers freeing non-substituted or branched xylooligosaccharides. Specific endoxylanases may only act on xylans with β-1,4 linkages. Non-specific endoxylanases are capable of hydrolyzing β-1,4-linked xylans, β-1,4 mixed xylans, and other β-1,4 polymers like carboxymethyl-cellulose for example (Paloheimo et al., 2010).

Pectinase is responsible for breaking down pectin which is primarily composed of homogalacturonan, arabinans, galactans, arabinogalactans, and rhamnogalacturonan I and II. Pectin, a polysaccharide found in plant cell walls, plays a role in plant structure, pH and ion balance, foreign molecule recognition, and cell wall porosity (Sticklen, 2008). Pectin makes up 1% of corn and 6% of soybean meal (Jackson, 2010).

Malathi and Devegowda (2001) conducted a two-stage in vitro digestion experiment utilizing xylanase, cellulase, and pectinase in broiler starter diets. They saw changes in sugar release and viscosity when supplementing these enzymes which improves nutrient utilization (Malathi and Devegowda, 2001). This is important because xylanase, cellulase, and pectinase

improved viscosity in the lumen which released more sugar in turn improving digestion and absorption.

Poultry lack the ability to effectively utilize natural fiber (fiber derived from plant sources) due to limited fermentation digesting enzymes. β-glucans are commonly found in barley, rye, triticale, and wheat and birds lack endogenous enzymes needed to break down these β-linked glucose polymers. Therefore, β-glucanases are added to diets in order to provide assistance in the breaking down of β-linked glucose polymers to oligosaccharides and glucose (Svihus, 2010). β-glucans pose problems on viscosity within the gut and often affect rate of passage which in turn decreases the rate of nutrient absorption (Svihus, 2010).

The enzyme α-amylase cleaves the α-linked bond of starch yielding glucose and maltose (Isaksen, 2010). Gracia et al. (2003) conducted a 42 d trial that utilized 168 male Cobb 500 chicks testing two dietary treatments (corn-soybean diet and corn-soybean diet with 1,720 units of α-amylase/kg). Daily gain and feed intake from 0-42 d was significantly increased by 2.6 g/d and 3.3 g/d, respectively, with the supplementation of α-amylase in the diet compared to the control (Gracia et al., 2003). This increase in feed intake may confound experimental responses of this experiment and may have been avoided if birds were on restricted feed intake. In this study, α-amylase increased feed intake which would mean adding a cost to the producer for feed. However, the broilers increased daily gain which would have beneficial impacts when birds are sold and processed. The economic gain, if any, would be dependent upon whether the increased bird weight overcame the increased feed input.

Amino acids are essential building blocks leading to lean muscle accretion in birds. In turn, proteases are added to poultry diets to enhance the breaking down of larger proteins into smaller peptides and amino acids for better absorption. The amount of available protein and quality varies

greatly among the raw materials found in poultry. Anti-nutritional factors, such as nonstarch polysaccharides and lectins, are found in soybean meal and other plant protein sources and can have detrimental effects on nutrient digestion and/or absorption because of their capability to damage the absorptive surface of the gut (Francis et al., 2001). The addition of proteases to poultry diets can assist in compensating the negative effects of the anti-nutritional factors and decrease nitrogen excretion (Isaksen, 2010).

Most enzymes can be used in combination with other enzymes in order to release more nutrients and be collectively beneficial to the bird. Scheideler et al. (2005) conducted a 15 wk experiment using a factorial arrangement in which corn-soy based diets were used to produce a normal ME or reduced ME diets (2,890 kcal/kg and 2,805 kcal/kg respectively) with or without an enzyme supplementation cocktail of xylanase, amylase, and protease (XAP). Two different strains of Single Comb White Leghorn hens (Babcock B-300 and Hy-Line W-36) were used when birds reached 25 wk of age. The enzyme cocktail significantly ($p < 0.03$) increased nitrogen retention almost 3%. There was a significant ($p < 0.04$) interaction on average hen-day egg production in which Hy-Line W36 laying hens performed better with the inclusion of enzyme potentially indicating any extra energy released from the enzyme went to the bird's production needs. There was a significant ($p < 0.01$) effect of dietary energy on AME_n in which the higher energy diet was 146 kcal/kg higher than the lower energy diet. Apparent metabolizable energy in excreta was not improved with enzyme supplementation which is surprising since the three enzymes should allow the hen more access to the nutrients increasing AME (Scheideler et al., 2005). The three enzymes together may not have had enough time to be able to act on their targeted substrates in the bird's short digestive tract. Bobeck and others (2014) have seen similar responses with xylanase.

Gunawardana and others (2009) conducted a 12 wk experiment in order to evaluate the effects of dietary energy and five enzyme activities (xylanase, mannanase, pectinase, protease, and β-glucanase) on performance of 1,920 Hy-Line W-36 second-cycle laying hens 87 wks of age. The experiment was set up as a factorial arrangement consisting of four dietary energy levels (2,791, 2,857, 2,923, and 2,989 kcal of ME/kg) and with or without enzyme cocktail. No significant effects were found for enzyme cocktail treatment on average egg weight. No significance was found with enzyme cocktail supplementation on feed intake however, as dietary energy increased feed intake decreased by 3.1% from 98 to 94.9 g/hen/d which has been demonstrated in a number of other experiments. Hens that consumed the highest energy level (2,989 kcal/kg) had significantly ($p < 0.01$) increased body weights by at least 100 g than hens on lower energy diets. Hens supplemented with enzyme cocktail had significantly ($p < 0.01$) increased body weights by 120 g than hens without enzyme supplementation (Gunawardana et al., 2009). Enzyme supplementation did not affect performance parameters but did however, influence body weight which is why accounting for body composition is important in these kinds of experiments.

Cheng et al. (2005) conducted a 20 wk laying hen experiment to determine the effect of a solid-state fermentation (SSF) product. In order to do this they added Allzyme® SSF (200 g/t) to a corn-soybean meal diet. This product is derived from the fungus *Aspergillus niger* and contains eight various enzyme activities that include phytase, xylanase, protease, cellulase, beta-glucanase, amylase, pentosanase, and pectinase. *Aspergillus niger* forms a natural enzyme complex when created by solid-substrate fermentation as compared to conventional liquid fermentation (Hooge et al., 2010). A total of 864 Hy-Line brown layers were utilized for this experiment from 21 to 41 wk of age. The three dietary treatments given included a positive control (2,700 kcal/kg), a negative control (2,600 kcal/kg), and the negative control diet plus SSF product and hens were

offered *ad libitum* access to feed. Dietary treatment had no significance on egg weight, egg production, feed intake, feed conversion ratio, weight gain, and mortality. The addition of SSF significantly ($p \leq 0.05$) improved AME by 184 kcal/kg (Cheng et al., 2005). Some limitations to this product may be the eight various enzyme activities. Also laying hens have short digestive tracts which may not allow sufficient time for all the activities to act. An advantage to using this product is the fact it has the potential to target different feedstuffs and release bound nutrients for the bird to utilize.

In summary, enzymes can decrease poultry feed costs by releasing bound nutrients allowing more meat or eggs produced per kilogram of feed (Barletta, 2010). These experiments captured metabolizable energy differences with the enzymes but few performance differences were detected so we need to consider fat storage due to the increase energy release. Different ways to measure laying hen body composition include body weights, abdominal fat pad, and dual-energy x-ray absorptiometry. Since body composition was not measured in the experiments conducted by Cheng (2005), Scheideler (2005), and Gunawardana (2009) the extra energy liberated from the enzyme may have been utilized for storage but we cannot be certain.

Mannan Oligosaccharide

Mannan oligosaccharides (MOS) are carbohydrate complexes composed of short chains of mannose sugars. Mannan oligosaccharides are derived from the yeast cell wall of *Saccharomyces cerevisiae* and they contain glucans, proteins, phosphate radicals, and mannose (Hajati and Rezaei, 2010). Mannan oligosaccharides are commonly used in the same manner as prebiotics with the exception of selectively enriching for beneficial bacterial populations (Patterson and Burkholder, 2003). Mannose is capable of inhibiting pathogenic bacteria, however, exact mechanisms have not been demonstrated (Hajati and Rezaei, 2010).

Sims et al. (2004) conducted an 18 wk experiment utilizing 720 Hybrid tom poults in which they tested the inclusion level of 0.10% MOS for the first 6 wk and 0.05% MOS thereafter. Results indicated a significant ($p < 0.05$) effect for feed conversion ratio (kg feed/kg live weight) in which the MOS treatment was at least 0.14 kg feed/kg bw more efficient than the control diet at 12, 15, and 18 wk (Sims et al., 2004). Mannan oligosaccharide (supplemented at 1 kg/ton until wk 6 and 0.5 kg/ton thereafter) in male turkey diets from 1-140 d of age improved body weight gain and feed conversion potentially due to the influence of nutrient utilization and possible nutrient sparing effect (Parks et al., 2001).

Kim et al. (2011) conducted a 4 wk experiment utilizing 240 Ross broiler chicks in which 0.1% or 0.2% MOS was included in the diet to evaluate performance differences. There was a significant ($p < 0.05$) effect of MOS on BW gain from wk 0-4 in which birds offered the diet supplemented 0.2% MOS diet had 31 g higher BW gain than did the control birds. Feed intake for birds wk 3-4 was significantly ($p < 0.05$) increased by 14-55 g in the 0.1% MOS diet compared to the control and 0.2% MOS diet (Kim et al., 2011). An experiment was conducted utilizing 2,835 day-old (Hubbard Hi-Y x Hubbard male) broiler chicks to test various inclusions of MOS. The three dietary treatments included a control diet (no added MOS), a basal diet plus MOS in starter, grower, and finisher (1.0, 0.5, and 0.5 kg/ton respectively), and a basal diet plus MOS in starter diet only (1.0 kg/ton). The chicks supplemented MOS in all three phases had significantly ($p < 0.05$) higher body weights at d 21, 28, 35, and 42 (22, 35, 61, and 57 g respectively) compared the control diet (Benites et. al, 2008). Zhang et al. (2005) conducted a 5 wk trial utilizing 240 Ross male broiler chicks in order to evaluate the effects of whole cell, cell wall, and cell content of *Saccharomyces cerevisiae* (SC) on growth performance. The 4 dietary treatments consisted of a control, 0.5% whole yeast (WY), 0.3% SC extract (YE), and 0.3% SC cell wall (CW). There was

a significant ($p = 0.05$) effect of SC on feed to gain ratio from wk 0-3 in which the CW treatment was improved by 0.13 g feed/g bw gain over the control diet and the WY and YE treatments were intermediate of those. From wk 4-5 a significant ($p = 0.05$) effect of SC on body weight were seen in which the WY and CW treatments were 74-83 g greater than the control diet. Another significant ($p = 0.05$) effect was seen during wk 4-5 on feed to gain ratio in which it improved by 0.12 g feed/g bw gain in the WY treatment over the control. From wk 0-5 body weight gain was significantly ($p = 0.05$) improved by 67-73 g in the WY and CW diets as compared to the control (Zhang et al., 2005).

Shashidhara and Devegowda (2003) conducted a 7 wk experiment using 3,120 Cobb broiler breeders (2,880 female and 240 males) which were 60 wk old at the beginning of the experiment. Broilers were offered a control diet or a diet supplemented with 0.50 g/kg of MOS and were on restricted feed intake in which females were fed 175 g/bird/d and males were fed 125 g/bird/d. There was a significant ($p < 0.05$) effect during week 60, 61, and 62 in which egg production was 1-3% higher in the MOS diet compared to the control. However, egg production during wk 63, 64, 65, and 67 was significantly ($p < 0.05$) increased in the control diet as compared to the MOS diet by 0.8-1.5%. There was no significant difference ($p < 0.05$) in egg production seen during wk 66 between the control and MOS diets (Shashidhara and Devegowda, 2003). These results may indicate that the MOS treatment works better at some stages of production than others or that the MOS has short term egg production effects.

Bozkurt et. al (2012) conducted a 16 wk experiment utilizing 432 commercial white laying hens, Lohmann LSL-classic, 36 wk old. Two diets (control and basal diet with 1 g/kg of MOS) were given over two periods (April to May and June to July) and hens were evaluated for performance and egg quality responses. No significant ($p < 0.05$) effects or interactions were found

between the diets and periods on performance parameters that included egg production, egg weights, egg mass, feed consumption, feed conversion ratio, and body weight (Bozkurt et al., 2012). Zaghini et al. (2005) conducted an experiment utilizing 96 Warren laying hens, 44 wk of age, to determine the effects of a corn-soy diet with an inclusion level of 0.11% mannan oligosaccharides (MOS). Egg weight decreased ($p < 0.05$) during wk 2 and 3 when hens were supplemented with 0.11% MOS as compared to the control diet (Zaghini et al, 2005). Yalcin et al. (2010) conducted a 16 wk experiment that utilized 225 Hy-Line Brown laying hens in order to evaluate dietary yeast autolysate on body weight, feed intake, hen-day egg production, egg weight, and feed efficiency. Hens were 22 weeks of age at the beginning of the experiment. The five experimental diets included a control and four levels (1, 2, 3, and 4 g/kg) of yeast autolysate (*Saccharomyces cerevisiae*). Yeast autolysates consist of both intracellular and cell wall fractions of ruptured or lysed cells. There were no significant ($p < 0.05$) differences seen within dietary treatments for body weight and feed intake. The addition of 2, 3, and 4 g/kg yeast autolysate significantly ($p < 0.001$) improved hen-day egg production 1-3% compared to the control and 1 g/kg diets. The addition of 1, 2, 3, and 4 g/kg of yeast autolysate significantly ($p < 0.001$) increased egg weight 0.7 g as compared to the control diet. Feed efficiency was significantly ($p < 0.03$) improved by 0.06 kg feed per kg egg when 2, 3, and 4 g/kg yeast autolysate was added to diets as compared to the control (Yalcin et al., 2010).

In summary, utilizing MOS in poults improved body weight gain and feed conversion and when added to broiler diets increased body weight gain. The addition of MOS seems important in young animals because they are naturally trying to establish microbial populations within the gastrointestinal tract while trying to grow. Mannan oligosaccharide increased egg production in broiler breeders for the first few weeks of the experiment. The use of MOS in laying hen diets has

been dependent upon the age of birds and stage of production. Some of the limitations noted in laying hen experiments may be due to not quantifying differences in energy. Therefore, the hypothesis is that various energy levels with the addition of MOS over a short-term experiment will allow for quantification of metabolizable energy in maintenance, production, and storage. Performance parameters will help measure both production and maintenance requirements and abdominal fat pad and DXA analysis will assist in measuring laying hen energy storage. Finally, nutrient digestibility will quantify the amount of metabolizable energy available to the bird.

Conclusions

Experiments conducted looking at varying dietary energy levels, MOS products, and enzyme supplementation have focused on performance and a handful of other parameters. However, body composition with the exception of Murugesan and Persia (2013) who looked at abdominal fat pad in laying hens has not been typically investigated. Since body composition was not measured in the majority of experiments with laying hens, questions remain if extra energy liberated from the enzyme or MOS is utilized for storage within the abdominal fat pad.

Therefore, the objectives of our experiments were to evaluate performance parameters, nitrogen corrected apparent metabolizable energy, and measure abdominal fat pad weights in combination with DXA analysis. In doing this we aim to quantify how much energy (if any) was spared by the MOS and enzymes and how those nutrients were utilized whether it be for maintenance, production, or storage over the experimental period.

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CHAPTER 3

EFFECTS OF A MANNOSE RICH FRACTION DERIVED FROM A SPECIFIC STRAIN OF YEAST OR AN ASPERGILLUS NIGER SOLID-STATE FERMENTATION PRODUCT ON PERFORMANCE, BODY COMPOSITION, AND NITROGEN CORRECTED APPARENT METABOLIZABLE ENERGY OF FIRST-CYCLE LAYING HENS FED TWO CONCENTRATIONS OF DIETARY ENERGY

Abstract

The objective of these experiments was to evaluate two feed additives, a mannose rich fraction (**MRF**) from a specific strain of yeast or an *Aspergillus niger* solid-state fermentation product (**SSF**) that contained various enzyme activities on performance, body composition, and nitrogen corrected apparent metabolizable energy (**AME_n**) utilizing Hy-Line W36 laying hens fed two dietary energy concentrations for a short period of time. The experiments were arranged as two 2 x 2 factorials, including diets with and without mannose rich fraction (Experiment 1) or solid-state fermentation (Experiment 2) and two concentrations of dietary energy (2,750 and 2,850 kcal/kg). Hens were housed individually (1,239 cm^2) for the 8 wk feeding period with a total of 12 hens for each of the 6 dietary treatments. Egg production, egg weight, egg mass, and mortality were recorded daily while feed intake was determined weekly. Excreta were collected during wk 8 for AME_n determination. Hens were euthanized and de-feathered for body composition determination. Data were analyzed using ANOVA with a 2 x 2 factorial arrangement and Fisher's LSD test was used to separate means if significant ($P \leq 0.05$). In Experiment 1, increasing energy, but not MRF, resulted in significantly increased total g and % of fat mass in the hens as determined by DXA analysis. Similar response to dietary energy was observed for abdominal fat pad weight, but significance was not achieved ($p = 0.12$). High dietary energy and MRF both resulted in a significant increase in determined AME_n. Overall, the MRF treatment increased AME_n but the

increased dietary energy did not appear to be stored as increased fat content of the hen. In Experiment 2 (solid-state fermentation) there was a significant SSF by dietary energy interaction ($p = 0.02$) on fat mass of hens in which SSF treatment in the reduced dietary energy diet resulted in increased fat mass, but with the opposite effect in the high energy diet. Both dietary energy and SSF resulted in a significant ($p < 0.01$) increase in AME_n as increasing dietary energy increased AME_n by 136 kcal/kg and SSF treatment increased AME_n by 43 kcal/kg. As expected, no differences in performance parameters including egg production, egg weight, and egg mass were detected in experiments 1 and 2. Therefore, these short-term laying hen experiments were able to capture changes in body composition and AME_n due to both feed additive and energy treatment, but performance parameters were non-responsive.

Key Words: body composition, dietary energy, laying hen, mannose rich fraction, solid-state fermentation

Introduction

Feed costs account for approximately 70% of total poultry production costs (Barletta, 2010). Feed ingredient pricing and availability in poultry diets is being influenced by today's fuel markets. An alternative to high energy costs may be the supplementation of feed additives to increase efficiency of dietary energy utilization.

Demonstrating the effectiveness of energy releasing feed additives has become a challenge to quantify in high efficiency laying hen breeds. D'Alfonso et. al (1996) demonstrated that various levels of dietary ME in DeKalb XL laying hen diets coincided with feed intake over a 7 wk experiment with no significant differences in egg production, egg mass, or body weight. Feed

intake linearly decreased with increasing dietary energy levels indicating that hens consume feed until there energetic demands were met. DeKalb XL laying hens are larger and less efficient than Hy-Line W36 hens with more ability to respond to changes in dietary energy by changing feed intake. In the Hy-Line W36, egg production was not affected by differences in dietary energy (Wu et. al, 2005; Harms et. al, 2000). The results of an experiment conducted by Murugesan and Persia (2013) suggests that energy in laying hens is partitioned in a particular pattern of production and maintenance before storage of lipid within the fat pad will occur. In a short-term 12 wk experiment utilizing Hy-Line W36 laying hens, they found the abdominal fat pad to be the most responsive measurement to dietary energy (Murugesan and Persia, 2013). Outside of a few experiments, body composition has not measured, therefore, it is difficult to be determined whether small differences in energy from differing dietary energy levels and/or feed additives was utilized for production, maintenance, or storage.

Mannan oligosaccharide (**MOS**) is essentially a carbohydrate complex composed of short chains of mannose sugars. Yalcin et al. (2010) utilized Hy-Line Brown laying hens to determine if a dietary yeast autolysate at inclusion levels of 2-4 g/kg in diets effected performance parameters over a 16 wk experimental period. They saw enhanced egg production, egg weight, feed efficiency, and humoral immune response (Yalcin et al., 2010). Bozkurt et. al (2012) found that Lohmann LSL-classic laying hens supplemented with 1 g MOS/kg in their diets had no significant ($p < 0.05$) effect on feed intake, feed conversion ratio, egg weight, and egg mass. These results provide contrasting results that may be due to differences in bird strain, MOS product, and/or MOS dose utilized.

Enzymes are added to laying hen diets to improve the nutritive value of various feedstuffs by increasing the availability of nutrients and driving efficiency (Barletta, 2010). Cheng and

coworkers (2005) found that Hy-Line Brown laying hens fed three dietary treatments that included a positive control (PC) containing 2,700 kcal/kg, negative control (NC) containing 2,600 kcal/kg, and NC + enzyme (Allzyme SSF) had no significant effects on egg weight, daily laying egg weight, feed intake, feed conversion ratio, or mortality rate over the 20 wk experiment, although, the AME for the NC + Allzyme SSF treatment was significantly greater ($p \leq 0.05$) compared to the NC diet alone. As noted above, fat storage is the most sensitive indicator of hen energy status and the changes in AME noted in the experiment might have altered fat content of the body before affecting body weight or egg production.

The objective of these experiments was to evaluate the addition of a feed additive, mannose rich fractions (**MRF**) or a solid-state fermentation (**SSF**) product on performance, body composition, abdominal fat pad, and nitrogen corrected apparent metabolizable energy (**AME_n**) of laying hens fed diets differing in energy concentration over a short-term 8 wk experiment.

Materials and Methods

The Institutional Animal Care and Use Committee of Iowa State University approved all animal procedures before initiation of the experiment. A total of 120 Hy-Line W-36 (Hy-Line International, Dallas Center, IA) laying hens were transferred from a local commercial facility at 22 wk of age. The hens were provided a 10 wk transition period during which they were fed a standard corn-soy diet prior to the start of the experiment. Experimental diets were administered at the beginning of wk 32 until the end of wk 40 (**Table 1**). Hens were provided *ad libitum* access to water and were offered to 95 g/d of feed. Feed intake was determined by Hy-Line W36 recommendations for the age of hens. Feed was limited to 95 g daily to try to reduce any potential differences in feed intake and ultimately energy intake. Experimental diets were arranged as two

2 x 2 factorials containing 6 dietary groups: high energy (**HE**), low energy (**LE**), HE + MRF, LE + MRF, HE + SSF, and LE + SSF. The HE diets were formulated to contain 2,850 kcal/kg of ME and the LE diets had 2,750 kcal/kg of ME. The mannose rich fraction, Actigen®, was extracted from the cell wall of a specific strain of yeast and added to the diets at a 0.4 g/kg inclusion rate. Allzyme® SSF, derived from the fungus *Aspergillus niger* and created by solid-state fermentation, was added to the diets at a 0.2 g/kg inclusion rate. Feed additive inclusion rates were recommended by the manufacturer (Alltech Incorporation, Nicholasville, KY). A basal diet was used for all experimental diet generation to minimize potential ingredient differences among experimental diets. The high energy diets contained 16.47% crude protein, 3.27% ether extract, 2.04% crude fiber, 11.70% moisture, and 12.77% crude ash as analyzed and the low energy diets contained 17.00% crude protein, 3.08% ether extract, 2.39% crude fiber, 11.07% moisture, and 12.50% crude ash, again on an analyzed basis. The crude protein, moisture, and crude ash were very similar among the two diets. As expected, ether extract was higher in the HE diet and crude fiber was lower in order to obtain the 100 kcal difference between the two diets. Titanium (**Ti**) dioxide, an inert dietary marker, was used for AME_n determination and was added to all diets at the rate of 0.50%.

An experimental unit (**EU**) was defined as an individual hen in a single-tier cage (1,239 cm^2); for a total of 12 EU per dietary treatment. Hens were housed individually in order to best quantify feed intake data and eliminate competition for feed. At the beginning of wk 32 hens were weighed for initial BW and assigned to cages utilizing a block design in which dietary treatments within each block were randomized. The hens were provided a 15 ½ L: 8 ½ D photoperiod and temperature between 21°C to 24°C during the experiment.

Data Collection

Experimental hens were monitored twice daily. Each morning within the same hour span hens were fed 95 g of feed and eggs were collected for hen-day egg production (**HDEP**). Feed intake and daily egg weights were measured and recorded weekly throughout the duration of the experiment. Feed intake was determined for each hen by quantifying feed refusal calculated by (initial bucket and feed weight + feed added over the week – bucket weight and remaining feed). Egg mass was calculated by (average weekly egg weight * HDEP / 100). Feed efficiency was calculated by (egg mass / feed intake * 1000). Initial BW was determined for each hen. Clean trays were placed under individual hen cages (EU) for 48 h before the end of wk 8. Excreta samples, free of feed and feathers, were collected and frozen at -20°C on the same day. At the end of wk 8, all hens were weighed and euthanized via carbon dioxide asphyxiation. Birds were de-feathered, weighed, and scanned utilizing dual-energy x-ray absorptiometry (**DXA**) to determine carcass fat, lean tissue, and ash (Hester et al., 2004). A validation of DXA techniques proved the accuracy of DXA measurements on body mass, lean tissue mass, and fat tissue mass and percentage (Swennen et. al, 2004). Upon completion of DXA the hens were dissected and abdominal fat pads (**AFP**) were collected and weighed.

Chemical Analysis

In total, 72 excreta samples (one collected from each EU) were dried at 65°C for 3 d (Jacobs et al., 2011) and ground through a 1.0-mm screen (Brinkmann Instruments Inc., Westbury, NY). Using a convection oven, feed samples were dried for 24 h at 100°C (Yamato Scientific America Inc., Santa Clara, CA) and then ground through a 0.5-mm screen (Brinkmann Instruments Inc., Westbury, NY). Both excreta and feed samples were tested for nitrogen concentration on a LECO

TruMac N Combustion Nitrogen Determinator (LECO Corporation, St. Joseph, MI). Gross energy was determined for feed and excreta using an adiabatic oxygen bomb calorimeter (Parr Instrument Co., Moline, IL). The titanium dioxide concentrations for feed and excreta samples were determined as described by Leone (1973). Excreta and feed samples were analyzed in duplicate for nitrogen, gross energy, and titanium dioxide concentrations. The calculations for AME_n utilized the methods of Scott et al. (1982) but titanium dioxide replaced the chromic oxide marker. The equation to determine nitrogen corrected apparent metabolizable energy was: AME_n = (Diet GE-((Excreta GE*Diet Ti/ Excreta Ti) + (8.22*(Diet N-(Excreta N*Diet Ti/Excreta Ti))))).

Statistical Analysis

Data were analyzed as a two-way ANOVA using General Linear Models procedure of SAS (SAS Institute Incorporation, Cary, NC). Statistical analysis for Experiment 1 was carried out as a 2 x 2 factorial design to detect interactions and main effects of dietary energy and MRF product. Statistical analysis for Experiment 2 was carried out as a 2 x 2 factorial design to detect interactions and main effects of dietary energy and SSF product. Fisher's least significant difference (LSD) test was used to separate means if significant ($p \leq 0.05$). The same controls were used for Experiments 1 and 2 because both experiments were run simultaneously.

Results and Discussion

Experiment 1

There were no mortalities or birds removed from Experiment 1. Results of Experiment 1 with differing energy levels and MRF are shown in **Table 2**. Individually caged hens were on limited (95 g/hen/d) feed intake throughout the duration of this experiment in an attempt to avoid overeating for energy among diets (D'Alfonso et. al, 1996; Harms et. al, 2000; Wu et. al, 2005)

and as seen by Jalal et al. (2006) when cage space for hens increased so did feed intake. Even though hens were on limited feed intake during this experiment the birds didn't consume all 95 g of feed which may have influenced the results. There were no significant effects of dietary energy or MRF treatment on feed intake, HDEP, egg weight, egg mass, and feed efficiency. There was a significant (p = 0.04) interaction of dietary energy and MRF on feed intake in which the birds on the HE – MRF diet consumed 2.5 g more feed per day than birds on the LE – MRF diet. These results contradict the findings of D'Alfonso and others (2006) in which feed intake linearly decreased with increasing dietary energy levels indicating that DeKalb XL hens consume feed until there energetic demands are met which is why it is believed another factor was affecting feed intake. There was no significant interactions of dietary energy and MRF treatment on HDEP, egg weight, egg mass, and feed efficiency. This 8 wk experiment may have been too short to capture egg mass differences with the various dietary treatments offered as agreed upon by previous authors looking at varying energy levels in laying hens (D'Alfonso et. al, 1996; Harms et. al, 2000; Wu et. al, 2005; Murugesan and Persia, 2013). The findings for feed efficiency are consistent with the 12 wk experiment conducted by Murugesan and Persia (2013) in which no significant differences were found in feed efficiency across diets. Harms and Russell (2004) conducted an 8 wk experiment utilizing Hy-Line W36 hens fed two various concentrations of energy 2,519 and 2,798 kcal/kg. Both feed intake and egg production remained unaltered (Harms and Russell, 2004).). Bohnsack et al. (2002) conducted a 12-week experiment using 560 Hy-Line W36 laying hens fed seven various levels of ME obtained by utilizing corn oil (2,783, 2,891, 2,996, and 3,089 kcal/kg) or poultry fat (2,881, 2,975, and 3,059 kcal/kg) at 0, 2, 4, and 6% inclusion levels and concluded that feed intake did not increase when dietary ME was reduced from 2,996 to 2,783 kcal/kg.

Results of DXA analysis for laying hen fat mass and fat percent across dietary regimes for Experiment 1 are shown in **Table 3**. There was a significant ($p = 0.03$) effect of dietary energy on fat mass in which the HE fed birds had 51 g more fat mass than the hens on the LE diet. There was a significant ($p = 0.02$) effect of dietary energy on fat percent in which HE diet fed birds had 3.4% more body fat than the LE birds. Although dietary energy did not significantly effect AFP ($p = 0.12$), the effect was similar to DXA measured fat mass in which the HE fed birds had an increased fat mass and fat percent than the birds on the LE diet. These results are consistent to those of Murugesan and Persia (2013) in which different dietary energy levels changed the amount of energy stored in the AFP over a 12 wk period. In both the previous and current experiments, it appears that body composition may be a more sensitive short-term response criterion than BW or HDEP. No interactions were observed between dietary energy and MRF on fat mass, fat percent, and abdominal fat pad. There were significant main effects of dietary energy and MRF treatment (no significant interactions) on AME_n values ($p \leq 0.01$). The HE diet was 142 kcal/kg higher than the LE diet and the MRF was 43 kcal/kg greater than the control. Nutrient digestibility was expected to be higher in the high energy fed birds since the diets were formulated to have approximately 100 kcal/kg difference. Since the MRF was 43 kcal/kg greater than the control it can be concluded that the MRF did release dietary energy for the laying hen although that energy was not used for egg production or storage.

Experiment 2

There were no mortalities or birds removed from Experiment 2. Effects of dietary energy levels and SSF treatment on parameters tested throughout the 8 wk (Experiment 2) are shown in **Table 4.** There were no significant effects of dietary energy or SSF on HDEP, egg weight, egg mass, and feed efficiency; this is consistent with a number of other short-term experiments (Jalal

et al., 2006; Wu et. al, 2005; Harms et. al, 2000). There was a significant ($p = 0.01$) effect of dietary energy on feed intake in which the hens fed the HE diets consumed approximately 2 g more feed than the birds on the LE fed birds. There was a significant ($p = 0.01$) effect of SSF on feed intake in which the SSF birds consumed almost 2 g more feed than the control birds. Again these differences were unexpected and may be due to reduced overall all feed intake in this experiment. Care must be taken to understand the effects of differences in feed intake and ultimately energy intake on responses due to both dietary energy and feed additive in this experiment. There was a significant ($p = 0.03$) interaction of dietary energy and SSF on HDEP in which LE + SSF treatment had nearly 5% lower egg production than the HE + SSF treatment with both diets without SSF treatment intermediate between these two. As a result of this there was a significant ($p = 0.03$) interaction of dietary energy and SSF on feed efficiency in which the birds fed the LE – SSF diet was 25 g/kg more efficient than the birds fed the LE + SSF diet, with time with both high energy diets, regardless of feed additive, intermediate. The interaction for feed efficiency was seen because birds fed the LE diet consumed less feed than the SSF fed birds but produced the same egg mass as the SSF group.

Results of DXA analysis for laying hen fat mass and fat percent across dietary treatments for Experiment 2 are shown in **Table 5.** There was an interaction ($p = 0.02$) for DXA analysis of fat mass as SSF treatment of the low energy diet resulted in increased fat mass, but the opposite was noted on the higher energy diet. This result was unexpected, but it is important to point out that this diet had the highest feed intake resulting in the highest egg production that could have used dietary energy for production rather than storage. No significant interactions or effects were seen in fat mass percent ($p = 0.07$) and AFP ($p = 0.11$) but the interaction terms however not significant did coincide with the fat mass values. Feed intake or the lack there of may have

impacted laying hen fat storage. If adequate nutrients are not ingested the bird may have enough for maintenance and production requirements however, none leftover for storage within the abdominal fat pad. If feed intake is significantly reduced the bird may not have enough nutrients to produce as many eggs or the egg mass may be reduced. There was a significant ($p \leq 0.01$) effect of dietary energy and SSF treatment on AME_n digestibility. The HE diet was 136 kcal/kg greater than that of the LE diet and the SSF treatment was 43 kcal/kg greater than the control.

In conclusion, since these experiments were focused on dietary energy differences it was most important to control feed intake. In both experiments, hens didn't consume all 95 g offered which may have influenced the results. An outside factor, such as a mycotoxin, may have been contributing to the lack of feed intake, decreased egg production, and decrease in body weight in these experiments.

Laying hens fed diets varying in dietary ME concentrations and the addition of feed additives were rather insensitive to short-term laying hen production parameters. Hens did seem to be more sensitive to short-term body composition changes as seen by DXA and AFP. Body composition should be measured in addition to performance parameters for short-term experiments. Nitrogen corrected apparent metabolizable energy results indicate that both feed additives (MRF and SSF) liberated more energy for the laying hen.

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Table 1. Ingredient composition, calculated chemical composition, and analyzed chemical composition of laying hen diets throughout the 8 wk experiment.

Diet	32 to 40 wk	
(% unless otherwise indicated)	HE[1]	LE[2]
Ingredient Composition		
Corn	61.61	59.24
Soybean meal (48% crude protein)	13.45	12.82
Dried distillers grains with solubles	10.00	15.00
Meat/bone meal	3.00	1.32
Soy oil	1.20	0.25
Salt	0.23	0.23
DL Methionine	0.08	0.07
Bio-Lys[3]	0.03	0.09
Large particle limestone	4.29	4.40
Small particle limestone	4.29	4.40
Dicalcium Phosphate	0.73	1.09
Choline chloride 60%	0.10	0.10
Vitamin and mineral premix[4]	0.50	0.50
TiO_2	0.50	0.50
Chemical Composition (calculated)		
ME (kcal/kg)	2,850	2,750
Crude protein	15.89	15.94
Calcium	3.80	3.80
Phosphorus	0.63	0.64
Avail Phosphorus	0.40	0.40
Crude fat	4.99	4.26
Crude fiber	2.71	2.95
Dig Met+Cys	0.56	0.57
Dig Lys	0.64	0.64
Dig Thr	0.53	0.53
Chemical Composition As-Fed (analyzed)		
Crude protein	16.47	17.00
Ether extract	3.27	3.08
Crude fiber	2.04	2.39
Moisture	11.70	11.07
Crude ash	12.77	12.50

[1]High energy (HE) contained 2,850 kcal/kg.
[2]Low energy (LE) contained 2,750 kcal/kg.
[3]L-lysine 50.7% - L-lysine sulphate produced by fermentation with *Corynebacterium glutamicum*.
[4] Provided per kg of diet: selenium, 88 ppm; vitamin A, 1,320,000 IU; vitamin D_3, 440,000 ICU; vitamin E, 2,860 IU; menadione, 176mg; vitamin B_{12}, 1.87 mg; biotin, 6.6 mg; choline, 71,500 mg; folic acid, 220 mg; niacin, 6,600 mg; pantothenic acid, 1,760 mg; pyridoxine, 176 mg; riboflavin, 880 mg; thiamine, 220 mg.
[5]Feed additives were added to basal diets.

Table 2. Effects of dietary energy, with or without a mannose rich fraction (MRF), on performance in laying hen diets from 32 to 40 wk of age, Experiment 1.

Item	Feed Intake g/hen/d	HDEP[1] %	Egg Wt g	Egg Mass g/hen/d	Egg:Feed[2] g/kg
Energy					
HE[3]	91.6	87.8	59.1	51.8	566
LE[4]	90.6	89.5	59.3	53.0	585
Pooled SEM	0.53	1.7	0.71	1.0	10.7
MRF[5] Trt					
Control	90.9	89.5	59.6	53.3	587
MRF	91.4	87.8	58.7	51.5	564
Pooled SEM	0.53	1.7	0.71	1.0	10.7
Energy x MRF Trt					
HE – MRF	92.1[a]	89.0	60.2	53.5	581
LE – MRF	89.6[b]	90.0	59.0	53.1	593
HE + MRF	91.1[ab]	86.6	57.9	50.1	551
LE + MRF	91.7[ab]	89.0	59.5	52.9	578
Pooled SEM	0.75	2.4	1.0	1.4	15.1
p-value					
Energy	0.20	0.48	0.84	0.39	0.21
MRF Trt	0.50	0.47	0.36	0.22	0.14
Energy x MRF	0.04	0.78	0.17	0.25	0.62

[a,b] In the same column least squares means not sharing a common superscript differ significantly, $p \leq 0.05$.

[1]Hen day egg production (HDEP).

[2]Egg mass produced per feed consumed.

[3]High energy (HE) contained 2,850 kcal/kg.

[4]Low energy (LE) contained 2,750 kcal/kg.

[5]Mannose rich fraction (MRF).

Table 3. Effects of dietary energy, with or without a mannose rich fraction (MRF), on abdominal fat pad (AFP) and nitrogen corrected apparent metabolizable energy (AME_n) in laying hen diets from 32 to 40 wk of age, Experiment 1.

Item	Fat Mass[1] G	Fat Mass[2] %	AFP[3] g	AME_n[4] kcal/kg
Energy				
HE[5]	357.1[a]	28.3[a]	35.6	3259[a]
LE[6]	306.1[b]	24.9[b]	29.5	3117[b]
Pooled SEM	15.88	0.98	2.75	8.3
MRF[7] Trt				
Control	330.2	27.0	32.6	3168[b]
MRF	333.0	26.3	32.5	3209[a]
Pooled SEM	15.88	0.98	2.75	8.3
Energy x MRF Trt				
HE – MRF	352.4	27.8	37.0	3239
LE – MRF	307.9	26.1	28.1	3096
HE + MRF	361.7	28.8	34.2	3279
LE + MRF	304.3	23.7	30.8	3139
Pooled SEM	22.46	1.41	3.89	11.7
p-value				
Energy	0.03	0.02	0.12	<0.01
MRF Trt	0.90	0.62	0.99	<0.01
Energy x MRF	0.77	0.23	0.48	0.90

[a,b] In the same column least squares means not sharing a common superscript differ significantly, $p \leq 0.05$.
[1]Fat mass in grams as determined by dual-energy x-ray absorptiometry (DXA).
[2]Fat mass percent as determined by dual-energy x-ray absorptiometry (DXA).
[3]Abdominal fat pad (AFP).
[4]Nitrogen corrected apparent metabolizable energy (AME_n).
[5]High energy (HE) contained 2,850 kcal/kg.
[6]Low energy (LE) contained 2,750 kcal/kg.
[7]Mannose rich fraction (MRF).

Table 4. Effects of dietary energy, with or without solid-state fermentation treatment, on performance in laying hen diets from 32 to 40 wk of age, Experiment 2.

Item	Feed Intake g/hen/d	HDEP[1] %	Egg Wt g	Egg Mass g/hen/d	Egg:Feed[2] g/kg
Energy					
HE[3]	92.6[a]	91.0	59.4	53.9	583
LE[4]	90.8[b]	88.6	59.7	52.7	580
Pooled SEM	0.46	1.1	0.65	0.44	4.3
SSF[5] Trt					
Control	90.9[b]	89.5	59.6	53.3	587
SSF	92.6[a]	90.1	59.4	53.4	576
Pooled SEM	0.46	1.1	0.65	0.44	4.3
Energy x SSF Trt					
HE – SSF	92.1	89.0[ab]	60.2	53.5	581[ab]
LE – SSF	89.6	90.0[ab]	59.0	53.1	593[a]
HE + SSF	93.1	93.0[a]	58.6	54.4	584[ab]
LE + SSF	92.1	87.2[b]	60.3	52.3	568[b]
Pooled SEM	0.65	1.5	0.92	0.62	6.1
p-value					
Energy	0.01	0.13	0.81	0.06	0.72
SSF Trt	0.01	0.70	0.83	0.91	0.09
Energy x SSF Trt	0.24	0.03	0.13	0.19	0.03

[a,b] In the same column least squares means not sharing a common superscript differ significantly, $p \leq 0.05$.

[1]Hen day egg production (HDEP).

[2]Egg mass produced per feed consumed.

[3]High energy (HE) contained 2,850 kcal/kg.

[4]Low energy (LE) contained 2,750 kcal/kg.

[5]Solid-state fermentation (SSF).

Table 5. Effects of dietary energy, with or without solid-state fermentation treatment, on abdominal fat pad (AFP) and nitrogen corrected apparent metabolizable energy (AME_n) in laying hen diets from 32 to 40 wk of age, Experiment 2.

Item	Fat Mass[1] g	Fat Mass[2] %	AFP[3] g	AME_n[4] kcal/kg
Energy				
HE[5]	323.2	26.1	34.9	3258[a]
LE[6]	327.8	27.1	33.1	3122[b]
Pooled SEM	13.68	1.04	3.05	7.7
SSF[7] Trt				
Control	330.2	27.0	32.6	3168[b]
SSF	320.8	26.3	35.5	3211[a]
Pooled SEM	13.68	1.04	3.05	7.7
Energy x SSF Trt				
HE – SSF	352.4[a]	27.8	37.0	3239
LE – SSF	307.9[ab]	26.1	28.1	3096
HE + SSF	293.9[b]	24.5	32.8	3276
LE + SSF	347.6[ab]	28.2	38.2	3147
Pooled SEM	19.35	1.51	4.32	11.1
p-value				
Energy	0.81	0.51	0.68	<0.01
SSF Trt	0.63	0.67	0.50	<0.01
Energy x SSF Trt	0.02	0.07	0.11	0.51

[a,b] In the same column least squares means not sharing a common superscript differ significantly, $p \leq 0.05$.
[1]Fat mass in grams as determined by dual-energy x-ray absorptiometry (DXA).
[2]Fat mass percent as determined by dual-energy x-ray absorptiometry (DXA).
[3]Abdominal fat pad (AFP).
[4]Nitrogen corrected apparent metabolizable energy (AME_n).
[5]High energy (HE) contained 2,850 kcal/kg.
[6]Low energy (LE) contained 2,750 kcal/kg.
[7]Solid-state fermentation (SSF).

CHAPTER 4

EFFECTS OF AN ASPERGILLUS NIGER SOLID-STATE FERMENTATION PRODUCT ON PERFORMANCE, BODY COMPOSITION, AND NITROGEN CORRECTED APPARENT METABOLIZABLE ENERGY OF FIRST-CYCLE LAYING HENS FED TWO CONCENTRATIONS OF DIETARY ENERGY

Abstract

The objective of this experiment was to evaluate two inclusion levels of an *Aspergillus niger* solid-state fermentation (**SSF**) product that contains various enzyme activities on performance, body composition, and nitrogen corrected apparent metabolizable energy (**AME_n**) utilizing first-cycle Hy-Line W36 laying hens fed various dietary energy concentrations for 16 wk. The experiment was arranged as a 2 x 3 factorial, including two concentrations of dietary energy (2,850 and 2,950 kcal/kg) and three SSF inclusions (0%, 0.2%, and 0.4%). Nine hens (413 cm^2/bird) were considered an experimental unit (**EU**) and there was a total of 14 EU for each of the 6 dietary treatments. Egg production, egg weight, egg mass, and mortality were recorded daily while feed intake was determined weekly. Feed efficiency was calculated weekly. Excreta was collected for 48 h during wk 16 for AME_n determination utilizing titanium dioxide as an inert dietary marker. Hens were sacrificed at wk 16 for body composition which included fat mass as determined by dual-energy x-ray absorptiometry (**DXA**) and abdominal fat pad (**AFP**) weight as a cumulative measure of body composition. Data were analyzed as a two-way ANOVA using General Linear Models procedure of SAS and Tukey's HSD test was used to separate means if significant ($p \leq 0.05$). A significant effect of SSF on HDEP ($p \leq 0.01$) was seen in which the 200 SSF and 400 SSF fed birds resulted in an approximately 1% decreased egg production compared to the hens without SSF supplementation. The solid-state fermentation product had a significant ($p = 0.02$) effect on egg mass in which birds fed the 200 SSF and 400 SSF diets produced

approximately one gram less egg mass daily mostly due to reduced egg production and not egg weight. The solid-state fermentation product also had a significant (p = 0.02) effect on feed efficiency, again due to reduced egg production in which laying hens fed the 200 SSF diet resulted in a 9 g/kg reduction in feed efficiency in comparison with the hens feed without SSF. There were significant ($p \leq 0.01$) effects of dietary energy on 16 wk fat mass and AFP in which the high energy (**HE**) birds had approximately 30 g more fat mass and 10 g more fat stored in the AFP than the low energy (**LE**) fed birds. There was a significant ($p \leq 0.01$) interaction of Energy x SSF on nitrogen corrected apparent metabolizable energy (**AME_n**) in which the difference between the LE and HE diets was reduced as SSF was increased in the diets, possibly suggesting that SSF treatment can liberate energy in the low energy diet with a dose-dependent relationship. Overall, the SSF treatment improved nitrogen corrected apparent metabolizable energy, but did result in a negatively impacted on performance, and did not influence body composition.

Key Words: body composition, dietary energy, laying hen, solid-state fermentation, performance

Introduction

Feed costs account for approximately 70% of total poultry production costs (Barletta, 2010). Although poultry are efficient converters of feed to gain, they cannot digest 15-25% of the feedstuffs they consume because of anti-nutritional factors that may hinder digestion and/or remain indigestible to the endogenous enzymes present within the bird (Paloheimo et al., 2010). Anti-nutritional factors present in corn and soybean meal can interfere with the bird's feed utilization and may affect health and production (Francis et al., 2001). The anti-nutrients in corn include nonstarch polysaccharides, phytic acid, enzyme inhibitors, and resistant starches. Soybean meal contains protease inhibitors, nonstarch polysaccharides, lectins, phytic acid, and saponins

(Cowieson, 2005; Francis et al., 2001). Feed additives assist in releasing these bound nutrients for laying hens to utilize. A solid-state fermentation (**SSF**) product derived from the fungus *Aspergillus niger* contains various enzyme activities that include: phytase, xylanase, protease, cellulase, beta-glucanase, amylase, pentosanase, and pectinase (Hooge et al., 2010). The solid-state fermentation product may help deactivate nonstarch polysaccharides, protease inhibitors, lectins, phytic acid, and saponins. Therefore, solid-state fermentation products used at varying dietary energy concentration levels may have differing effects on hen production, body composition, and nitrogen corrected apparent metabolizable energy.

Laying hens utilize dietary energy in three ways: maintenance, production, and storage. D'Alfonso et. al (1996) demonstrated that various concentrations of dietary ME (2,580, 2,814, and 3,009 kcal/kg) in De-Kalb XL laying hen diets coincides with feed intake over a 7 wk experiment with no significant differences in egg production, egg mass, and body weight. An 8 wk experiment was performed in which Hy-Line W36 hens approximately 80 wks of age were fed two various concentrations of energy, 2,519 and 2,798 kcal/kg. Results indicated that laying hens feed intake and egg production remained unaltered (Harms and Russell, 2004). Egg production was not affected by differences in dietary energy over short-term experiments (Wu et. al, 2005; Harms et. al, 2000). No significant differences were found for production parameters over short-term experiments. Jalal et al. (2006) conducted a 15 wk experiment utilizing Hy-Line W36 laying hen looking at three dietary energy levels (2,800, 2,850, and 2,900 kcal/kg) and their effects on AME_n. They found that hens fed the 2,900 kcal/kg diet had significantly greater AME_n as compared to the 2,850 and 2,800 kcal/kg diets with differences of 107 and 118 kcal/kg, respectively. Nitrogen corrected apparent metabolizable energy differences were detected in short-term experiments with energy dietary energy differences.

Laying hen production only accounts for part of the energetic balance of the laying hen, therefore performance responses alone are not sufficient to quantify complete energy balance (Murugesan and Persia, 2013). The energy intake differences between the control (2,880 kcal/kg) and low ME (2,790 kcal/kg) diets did not alter hen production or maintenance but did reduce the energy stored in the fat pad of the hens on the lower ME diet. These results indicate that over a short-term experiment Hy-Line W36 laying hen's first partition energy to egg production and maintenance then storage as fat within the body (Murugesan and Persia, 2013).

A 15 wk experiment using two dietary ME concentrations (2,890 kcal/kg and 2,805 kcal/kg) and enzyme supplementation (xylanase, protease, and amylase). Apparent metabolizable energy was not improved with enzyme supplementation (Scheideler et al., 2005). A 12 wk experiment which contained four dietary energy levels (2,791, 2,857, 2,923, and 2,989 kcal of ME/kg) and five enzyme activities (xylanase, mannanase, pectinase, protease, and β-glucanas). Enzyme supplementation had no effect on egg weight, egg production, and feed intake, however hens gained weight (Gunawardana et al., 2009). Cheng et al. (2005) conducted an experiment to determine the effect of a solid-state fermentation product containing eight various enzyme activities (phytase, xylanase, protease, cellulase, beta-glucanase, amylase, pentosanase, and pectinase) and two dietary energy levels (2,600 and 2,700 kcal/kg) over 20 wk. Dietary treatment had no effect on egg weight, egg production, feed intake, feed conversion ratio, weight gain, and mortality, but did improve AME (Cheng et al., 2005). Since body composition was not measured in the previous enzyme experiments, it is possible that energy liberated from the enzyme treatment was not quantified as increased body fat content.

The objective of this experiment was to evaluate two inclusion levels of an *Aspergillus niger* solid-state fermentation product, Allzyme SSF, on performance, body composition,

abdominal fat pad, and nitrogen corrected apparent metabolizable energy of laying hens fed diets differing in energy concentration over a 16 wk experiment.

Materials and Methods

The Institutional Animal Care and Use Committee of Iowa State University approved all animal procedures before initiation of the experiment. A total of 756 Hy-Line W-36 (Hy-Line International, Dallas Center, IA) laying hens were transferred from a local commercial facility at 18 wk of age. The hens were provided a 17 wk transition period during which they were fed a standard commercial diet prior to the start of the experiment. At the beginning of wk 35 laying hens were weighed for initial BW and assigned to cages utilizing a block design in which dietary treatments within each block were randomized. Nine hens (413 cm^2/bird) represented 1 experimental unit (**EU**) and there was a total of 14 EU for each of the 6 dietary treatments. The hens were provided with a 15 ½ L: 8 ½ D photoperiod and temperature between 21°C to 24°C during experiment.

Experimental diets were administered for 16 wk when laying hens were 35 wk of age until 51 wk of age (**Table 1**). Hens were provided *ad libitum* access to water and were limited to approximately 95 g/hen/d of feed. Laying hens were on limited feed intake throughout the experiment in order to control energy intake and ensure that feed intake did not influence the results of other parameters measured. The experiment was arranged as a 2 x 3 factorial, including two concentrations of dietary energy (high energy and low energy) and three SSF inclusions (0%, 0.2%, and 0.4%). The high energy (**HE**) diets were formulated to contain 2,950 kcal/kg of ME and the low energy (**LE**) diets 2,850 kcal/kg of ME. The high energy diets contained 17.86% crude protein, 6.09% ether extract, 3.34% crude fiber, 9.28% moisture, and 14.66% crude ash and the

low energy diets contained 17.55% crude protein, 4.42% ether extract, 3.46% crude fiber, 9.43% moisture, and 14.55% crude ash. The crude protein, moisture, and crude ash were similar among the two diets. In order to obtain the 100 kcal difference between the two diets, soy oil was added to the high energy diet. The solid-state fermentation product, Allzyme® SSF, is derived from the fungus *Aspergillus niger* and created by solid-state fermentation. Feed additive inclusion rates were recommended by the manufacturer (Alltech Incorporation, Nicholasville, KY). Titanium (**Ti**) dioxide, an inert dietary marker used for AME_n determination, was added to all diets at the rate of 0.40%.

Data Collection

Experimental hens were monitored twice daily. Eggs were collected and weighed for hen-day egg production (**HDEP**) each morning. Hens were fed approximately 95 g/hen/d each afternoon. Feed intake was measured and recorded weekly throughout the experiment. Feed intake was determined for each hen by quantifying feed refusal calculated by (initial bucket and feed weight + feed added over the week – bucket weight and remaining feed). Egg mass was calculated by (average weekly egg weight * HDEP / 100). Feed efficiency was calculated by (egg mass / feed intake * 1000). Body weight was determined for each cage of hens at wk 0, wk 8, and wk 16. In order to prevent cross contamination of excreta samples, clean trays were placed under the middle cage of each EU for 48 h during wk 16. Excreta samples, free of feed and feathers, were collected and frozen at -20°C on the same day. At the end of wk 16, the three hens from the middle cage of each EU (234 birds total) were weighed and euthanized via carbon dioxide asphyxiation. All three hens from the same cage were removed to ensure that a representative sample of bird size was obtained for body competition and to be consistent among each EU. Birds were then de-feathered,

weighed, and scanned utilizing dual-energy x-ray absorptiometry (**DXA**). A validation of DXA techniques was completed for body mass, lean tissue mass, and fat tissue mass and percentage for laying hens (Swennen et al., 2004). Dual-energy x-ray absorptiometry has the capability to help quantify where a laying hen is utilizing these nutrients over the experimental period. Upon completion of DXA, hens were dissected and abdominal fat pads (**AFP**) were collected and weighed.

Chemical Analysis

Excreta samples were dried at 65°C for 3 d (Jacobs et al., 2011) and ground through a 1.0-mm screen (Brinkmann Instruments Inc., Westbury, NY). Using a convection oven feed samples were dried for 24 h at 100°C (Yamato Scientific America Inc., Santa Clara, CA) and then ground through a 0.5-mm screen (Brinkmann Instruments Inc., Westbury, NY). Excreta and feed were tested for nitrogen concentration on a LECO TruMac N Combustion Nitrogen Determinator (LECO Corporation, St. Joseph, MI). Gross energy was determined for feed and excreta using an adiabatic oxygen bomb calorimeter (Parr Instrument Co., Moline, IL). The titanium dioxide concentrations for feed and excreta samples were determined as described by Leone (1973). The calculation of AMEn utilized the methods of Scott et al. (1982) but titanium (**Ti**) dioxide replaced chromic oxide as an indigestible dietary marker. The equation to determine nitrogen corrected apparent metabolizable energy: AME_n = (Diet GE-((Excreta GE*Diet Ti/ Excreta Ti) + (8.22*(Diet N-(Excreta N*Diet Ti/Excreta Ti))))). Both excreta and feed samples were tested in duplicate for nitrogen, gross energy, and titanium dioxide.

Statistical Analysis

Data were analyzed as a two-way ANOVA using General Linear Models procedure of SAS (SAS Institute Incorporation, Cary, NC). Statistical analysis for nitrogen corrected apparent

metabolizable energy and body composition was carried out as a 2 x 3 factorial to detect interactions and main effects of dietary energy and SSF. Repeated measures were used to analyze performance parameters, but not body composition or AME_n. Tukey's HSD test was used to separate means if significant ($p \leq 0.05$).

Results and Discussion

There were two hen mortalities throughout the duration of the experiment. Hen mortality was accounted for in performance calculations. The results of the performance parameters for two dietary energy levels and three inclusion levels of SSF are shown in **Table 2**. There were no significant effects of dietary energy on feed intake, HDEP, egg mass, egg weight, and feed efficiency. This is consistent with other laying hen short-term experiments in which altering dietary energy did not affect performance parameters (Murugesan and Persia, 2013; Valkonen et. al, 2008; Wu et. al, 2005). A significant effect of SSF on HDEP ($p \leq 0.01$) was seen in which the 200 SSF and 400 SSF fed birds resulted in an approximate 1% decrease in egg production compared to the hens without SSF. The solid-state fermentation product had a significant ($p = 0.02$) effect on egg mass in which birds fed the 200 SSF and 400 SSF diets produced approximately one gram less mass daily than the hen without SSF due to reduced egg production and not egg weight. The solid-state fermentation product also had a significant ($p = 0.02$) effect on feed efficiency, again due to reduced egg production in which laying hens fed the 200 SSF diet resulted in a 9 g/kg reduction in feed efficiency in comparison with the hens feed without SSF. These results indicate that the SSF product did not improve, but rather decreased HDEP, egg mass, and feed efficiency throughout the 16 wk experiment. No significant interactions were found for Energy x SSF on feed intake, HDEP, egg mass, egg weight, and feed efficiency. These results are

consistent with other experiments in which there were various dietary energy and enzyme concentrations (Cheng et. al, 2005; Gunawardana et. al, 2009).

The results of two dietary energy levels and three inclusion levels of solid-state fermentation are shown in **Table 3** for body composition and nitrogen corrected apparent metabolizable energy in laying hen diets for wk 1-16. There were no significant effects or interactions of dietary energy and SSF on fat percent and lean mass. There was a significant ($p < 0.01$) effect of energy on fat mass for wk 1-16 in which the HE birds had approximately 30 g more fat mass than the LE fed birds. There was a significant ($p < 0.01$) effect of energy on AFP in which the HE birds had nearly 10 g more fat stored in the AFP compared to the LE fed birds. These results were expected because the HE birds were given 100 kcal/kg more energy than the LE birds and once maintenance and production energy requirements are met the excess energy will be stored as fat. There was a significant ($p \leq 0.01$) interaction of Energy x SSF on nitrogen corrected apparent metabolizable energy (**AME_n**) in which the difference between the LE and HE diets was reduced as SSF was increased in the diets, possibly suggesting that SSF treatment can liberate energy in the low energy diet with a dose-dependent relationship. In conclusion, dietary energy had no impact on performance or body weight, but did change body composition in response to changes in dietary nitrogen corrected apparent metabolizable energy. The addition of SSF decreased bird performance, improved AME_n, and had no effect on body composition.

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Table 1. Ingredient composition, calculated chemical composition, and analyzed chemical composition of laying hen diets throughout the 16 wk experiment.

Diet	35 to 51 wk of age	
(% unless otherwise indicated)	HE[1]	LE[2]
Ingredient Composition		
Corn	54.94	57.01
Soybean meal 48	20.61	20.41
Dried distillers grains with solubles	5.00	5.00
Meat/bone meal	4.00	4.00
Soy oil	4.04	2.18
Salt	0.33	0.33
DL Methionine	0.18	0.18
Large particle limestone	4.79	4.80
Small particle limestone	4.79	4.80
Dicalcium Phosphate	0.31	0.30
Choline chloride 60%	0.10	0.10
Vitamin and mineral premix[3]	0.50	0.50
TiO_2	0.40	0.40
Phytase	0.00075	0.00075
Chemical Composition (calculated)		
ME (kcal/kg)	2,950	2,850
Crude protein	17.96	18.01
Calcium	4.20	4.20
Phosphorus	0.58	0.59
Avail Phosphorus	0.36	0.36
Crude fat	7.14	5.44
Crude fiber	2.45	2.49
Dig Met+Cys	0.76	0.76
Dig Lys	0.89	0.89
Dig Thr	0.67	0.67
Chemical Composition As-Fed (analyzed)		
Crude protein	17.86	17.55
Ether extract	6.09	4.42
Crude fiber	3.34	3.46
Moisture	9.28	9.43
Crude ash	14.66	14.55

[1]High energy (HE) contained 2,950 kcal/kg.
[2]Low energy (LE) contained 2,850 kcal/kg.
[3] Provided per kg of diet: selenium, 88 ppm; vitamin A, 1,320,000 IU; vitamin D_3, 440,000 ICU; vitamin E, 2,860 IU; menadione, 176mg; vitamin B_{12}, 1.87 mg; biotin, 6.6 mg; choline, 71,500 mg; folic acid, 220 mg; niacin, 6,600 mg; pantothenic acid, 1,760 mg; pyridoxine, 176 mg; riboflavin, 880 mg; thiamine, 220 mg.

Table 2. Effects of dietary energy, with or without a solid-state fermentation product, on performance in laying hen diets from 35 to 51 wk of age.

Item	Feed Intake g/hen/d	HDEP[1] %	Egg Wt G	Egg Mass g/hen/d	Egg:Feed[2] g/kg
Energy					
HE[3]	96.3	91.0	62.0	56.2	584
LE[4]	96.2	90.5	61.9	55.8	580
Pooled SEM	0.11	0.23	0.13	0.20	1.8
SSF[5]					
Control	96.4	91.8[a]	61.9	56.6[a]	587[a]
200 SSF	96.4	90.2[b]	62.2	55.7[b]	578[b]
400 SSF	96.0	90.3[b]	61.8	55.7[b]	581[ab]
Pooled SEM	0.14	0.29	0.16	0.24	2.2
Energy x SSF					
HE – SSF	96.5	91.9	62.1	56.6	587
LE – SSF	96.4	91.7	61.7	56.6	587
HE + 200 SSF	96.6	90.8	62.3	56.3	583
LE + 200 SSF	96.2	89.6	62.1	55.2	574
HE + 400 SSF	95.9	90.4	61.7	55.7	581
LE + 400 SSF	96.1	90.2	61.8	55.7	580
Pooled SEM	0.20	0.41	0.22	0.34	3.1
p-value					
Energy	0.54	0.12	0.47	0.17	0.18
SSF	0.07	<0.01	0.13	0.02	0.02
Energy x SSF	0.29	0.46	0.42	0.19	0.24

[a,b] In the same column least squares means not sharing a common superscript differ significantly, $p \leq 0.05$.

[1]Hen day egg production (HDEP).

[2]Egg mass produced per feed consumed.

[3]High energy (HE) contained 2,950 kcal/kg.

[4]Low energy (LE) contained 2,850 kcal/kg.

[5]Solid-state fermentation (SSF).

Table 3. Effects of dietary energy, with or without a solid-state fermentation product, on abdominal fat pad (AFP) and nitrogen corrected apparent metabolizable energy (AME_n) in laying hen diets from experimental period wk 1-16.

Item	Fat Mass[1] G	Fat Percent[2] %	AFP[3] G	Lean Mass g	AME_n[4] kcal/kg
Energy					
HE[5]	372.1[a]	25.9	49.2[a]	1027.6	2973[a]
LE[6]	342.3[b]	24.7	39.3[b]	1006.4	2878[b]
Pooled SEM	8.2	0.44	1.7	9.9	7.3
SSF[7] Trt					
Control	348.7	25.0	43.8	1015.5	2899[b]
200 SSF	356.9	25.3	42.8	1015.4	2937[a]
400 SSF	365.9	25.7	46.3	1020.1	2941[a]
Pooled SEM	10.0	0.54	2.1	12.1	9.0
Energy x SSF					
HE – SSF	364.2	25.3	49.2	1043.1	2944[b]
LE – SSF	333.2	24.6	38.4	987.8	2854[d]
HE + 200 SSF	363.8	25.4	47.9	1031.3	3009[a]
LE + 200 SSF	350.0	25.2	37.7	999.5	2865[cd]
HE + 400 SSF	388.1	27.0	50.6	1008.2	2967[ab]
LE + 400 SSF	343.7	24.3	42.0	1031.9	2915[bc]
Pooled SEM	14.2	0.77	3.0	17.2	12.7
p-value					
Energy	0.01	0.06	<0.01	0.14	<0.01
SSF	0.48	0.65	0.50	0.95	<0.01
Energy x SSF	0.56	0.23	0.93	0.07	<0.01

[a,b] In the same column least squares means not sharing a common superscript differ significantly, $p \leq 0.05$.

[1]Fat mass in grams as determined by dual-energy x-ray absorptiometry (DXA).

[2]Fat percent as determined by dual-energy x-ray absorptiometry (DXA).

[3]Abdominal fat pad (AFP).

[4]Nitrogen corrected apparent metabolizable energy (AME_n).

[5]High energy (HE) contained 2,950 kcal/kg.

[6]Low energy (LE) contained 2,850 kcal/kg.

[7]Solid-state fermentation (SSF).

CHAPTER 5

GENERAL CONCLUSIONS

The first objective of the research project was to evaluate the addition of a feed additive, mannose rich fractions or a solid-state fermentation product, on performance, body composition, and nitrogen corrected apparent metabolizable energy of laying hens fed diets containing either 2,750 kcal/kg or 2,850 kcal/kg dietary energy over a short-term 8 wk experiment. The second objective of this research was to evaluate two inclusion levels of a solid-state fermentation product on performance, body composition, and nitrogen corrected apparent metabolizable energy of laying hens fed diets containing either 2,850 kcal/kg or 2,950 kcal/kg dietary energy over a 16 wk experiment.

Even though hens were on limited feed intake during 8 wk experiments 1 and 2 the birds didn't consume all 95 g of feed offered which may have influenced some of the results. Since these experiments were focused on dietary energy differences, differences in feed intake can change energy intake making interpretation of these data more complex.

Laying hens fed diets varying in dietary ME concentrations and the addition of feed additives over 8 wk were rather insensitive to short-term laying hen production parameters. Hens did seem to be more sensitive to short-term body composition changes as seen by DXA and AFP. Body composition should be looked at over performance parameters for short-term experiments. Nitrogen corrected apparent metabolizable energy results indicate that both feed additives (MRF and SSF) liberated more energy for the laying hen to utilize. Over the 16 wk experiment, dietary energy and SSF together had no impact on performance or body composition but did influence AME_n digestibility. The addition of SSF decreased bird performance, improved AME_n, and had

no effect on body composition. The dietary energy differences had no effect on performance parameters but did influence body composition and AME_n.

Overall, this research did fill a missing link in short-term laying hen experiments between looking at performance parameters, body composition, and nitrogen corrected apparent metabolizable energy. This research illustrates how important it is to look at body composition and AME_n collectively in short-term laying hen experiments. To bridge gaps left in the literature utilizing laying hens as a model different lengths of time, energy differences, heat loss, feed additives, and feed additive doses should still be further explored.